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(54) Title: N-(AMINO ACID) SUBSTITUTED SUCCINIC ACID AMIDE DERIVATIVES AS METALLOPROTEINASE INHIBITORS

(57) Abstract

Compounds selected from the group consisting of 2,N^1-dihydroxy-3-isobutyl-N^4-[2-methoxy-2-methyl-1-(pyridin-2-ylcarbamoyl)-propyl]-succinamide, N^1-(2-fluoro-2-methyl-1-methylcarbamoyl-propyl)-N^4-hydroxy-2-isobutyl-3-methoxy-succinamide, N^4-hydroxy-2-isobutyl-N^1-(2-mercapto-2-methyl-1-methylcarbamoyl-propyl)-3RS-methoxy-succinamide, 2-allyl-N^4-(2-fluoro-2-methyl-1-methylcarbamoyl-propyl)-N^1-hydroxy-3-isobutyl-succinamide, 2-allyl-N^1-hydroxy-3-isobutyl-N^4-(2-methyl-1-methylcarbamoyl-2-phenyl-propyl)-succinamide, 2-allyl-N^4-(2,2-dimethyl-1-methylcarbamoyl-but-3-enyl)-N^1-hydroxy-3-isobutyl-succinamide, 3-(3-hydroxycarbamoyl-2-isobutyl-hex-5-enoylamino)-2,2,N-trimethyl-succinamic acid methyl ester, 3-[2,2-dimethyl-1-(pyridin-2-ylcarbamoyl)-propylcarbamoyl]-2-hydroxy-5-methyl-hexanoic acid, and salts hydrates or solvates thereof, are matrix metalloproteinase inhibitors and inhibitors of the release of TNF-alpha.

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N-(AMINO ACID) SUBSTITUTED SUCCINIC ACID AMIDE DERIVATIVES AS METALLOPROTEINASE INHIBITORS

The present invention relates to therapeutically active hydroxamic acid and carboxylic acid derivatives, to pharmaceutical compositions containing them, and to the use of such compounds in medicine. In particular, the compounds are inhibitors of metalloproteinases involved in tissue degradation, and in addition are inhibitors of the release of tumour necrosis factor from cells.

Background to the Invention

Compounds which have the property of inhibiting the action of metalloproteinases involved in connective tissue breakdown such as collagenase, stromelysin and gelatinase (known as "matrix metalloproteinases", and herein referred to as MMPs) are thought to be potentially useful for the treatment or prophylaxis of conditions involving such tissue breakdown, for example rheumatoid arthritis, osteoarthritis, osteopenias such as osteoporosis, periodontitis, gingivitis, corneal epidermal or gastric ulceration, and tumour metastasis, invasion and growth. MMP inhibitors are also of potential value in the treatment of neuroinflammatory disorders, including those involving myelin degradation, for example multiple sclerosis, as well as in the management of angiogenesis dependent diseases, which include arthritic conditions and solid tumour growth as well as psoriasis, proliferative retinopathies, neovascular glaucoma, ocular tumours, angiofibromas and hemangiomas.

Tumour necrosis factor (herein referred to as "TNF") is a cytokine which is produced initially as a cell-associated 28kD precursor. It is released as an active, 17kD form , which can mediate a large number of deleterious effects in vivo. When administered to animals or humans it causes inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase responses, similar to those seen during acute infections and shock states. Chronic administration can also cause cachexia and anorexia. Accumulation of excessive TNF can be lethal.

There is considerable evidence from animal model studies that blocking the effects

of TNF with specific antibodies can be beneficial in acute infections, shock states, graft versus host reactions and autoimmune disease. TNF is also an autocrine growth factor for some myelomas and lymphomas and can act to inhibit normal haematopoiesis in patients with these tumours.

Compounds which inhibit the production or action of TNF are therefore thought to be potentially useful for the treatment or prophylaxis of many inflammatory, infectious, immunological or malignant diseases. These include, but are not restricted to, septic shock, haemodynamic shock and sepsis syndrome, post ischaemic reperfusion injury, malaria, Crohn's disease, mycobacterial infection, meningitis, psoriasis, congestive heart failure, fibrotic disease, cachexia, graft rejection, cancer, autoimmune disease, rheumatoid arthritis, multiple sclerosis, radiation damage, toxicity following administration of immunosuppressive monoclonal antibodies such as OKT3 or CAMPATH-1 and hyperoxic alveolar injury.

Since excessive TNF production has been noted in several diseases or conditions also characterised by MMP-mediated tissue degradation, compounds which inhibit both MMPs and TNF production may have particular advantages in the treatment or prophylaxis of diseases or conditions in which both mechanisms are involved.

Recently, WO 93/20047 disclosed a class of hydroxamic acid based MMP inhibitors which also are active in inhibiting TNF production.

As mentioned above, MMP inhibitors have been proposed with hydroxamic acid or carboxylic acid zinc binding groups. The following patent publications disclose hydroxamic acid-based and/or carboxylic acid-based MMP inhibitors:

US 4599361	(Searle)
EP-A-2321081	(ICI)
EP-A-0236872	(Roche)

EP-A-0274453	(Bellon)
WO 90/05716	(British Bio-technology)
WO 90/05719	(British Bio-technology)
WO 91/02716	(British Bio-technology)
WO 92/09563	(Glycomed)
US 5183900	(Glycomed)
US 5270326	(Glycomed)
WO 92/17460	(SmithKline Beecham)
EP-A-0489577	(Celltech)
EP-A-0489579	(Celltech)
EP-A-0497192	(Roche)
US 5256657	(Sterling Winthrop)
WO 92/13831	(British Bio-technology)
WO 92/22523	(Research Corporation Technologies)
WO 93/09090	(Yamanouchi)
WO 93/09097	(Sankyo)
WO 93/20047	(British Bio-technology)
WO 93/24449	(Celltech)
WO 93/24475	(Celltech)
EP-A-0574758	(Roche)
EP-A-0575844	(Roche)
WO 94/02447	(British Biotech)
WO 94/02446	(British Biotech)
WO 94/21612	(Otsuka)
WO 94/21625	(British Biotech)
WO 94/24140	(British Biotech)
WO 94/25434	(Celltech)
WO 94/25435	(Celltech)
WO 95/04033	(Celltech)
WO 95/04735	(Syntex)
WO 95/04715	(Kanebo)
WO 95/09841	(British Biotech)

WO 95/12603 (Syntex)

In addition, disclose hydroxamic acid- and carboxylic acid based MMP inhibitors principally characterised by the identity of the groups corresponding to R

Brief Description of the Invention

The present invention makes available certain MMP inhibitors falling within the general disclosure of our copending International Patent Applications nos PCT/GB95/00111 and PCT/GB95/00121, which have the general properties therein described, but which are not specifically disclosed or characterised therein.

Detailed Description of the Invention

The present invention provides compounds selected from the group consisting of:

2,N^1-Dihydroxy-3-isobutyl-N^4-[2-methoxy-2-methyl-1-(pyridin-2-yl-carbamoyl)-propyl]-succinamide,

N^1-(2-Fluoro-2-methyl-1-methylcarbamoyl-propyl)-N^4-hydroxy-2-isobutyl-3-methoxy-succinamide,

N^4-Hydroxy-2-isobutyl-N^1-[2-mercapto-2-methyl-1-methylcarbamoyl-propyl)-3RS-methoxy-succinamide,

2-Allyl-N^4-(2-Fluoro-2-methyl-1-methylcarbamoyl-propyl)-N^1-hydroxy-3-isobutyl-succinamide,

2-Allyl-N^1-hydroxy-3-isobutyl-N^4-(2-methyl-1-methylcarbamoyl-2-phenyl-propyl)-succinamide,

2-Allyl-N^4-(2,2-dimethyl-1-methylcarbamoyl-but-3-enyl)-N^1-hydroxy-3-isobutyl-succinamide,

3-(3-Hydroxycarbamoyl-2-isobutyl-hex-5-enoylamino)-2,2,N-trimethyl-succinamic acid methyl ester,

3-[2,2-Dimethyl-1-(pyridin-2-ylcarbamoyl)-propylcarbamoyl]-2-hydroxy-5-methyl-hexanoic acid,

and salts hydrates or solvates thereof.

In the compounds of the invention, the preferred stereochemistry is in general as follows:

C atom carrying the R_1 and X group - S,

C atom carrying the R_2 group - R,

C atom carrying the R_3 group - S,

but mixtures in which the above configurations predominate are also contemplated.

Accordingly the preferred stereoisomers of the compounds of the invention are:

2S,N^1-Dihydroxy-3R-isobutyl-N^4-[2-methoxy-2-methyl-1S-(pyridin-2-yl-carbamoyl)-propyl]-succinamide,

N^1-(2-Fluoro-2-methyl-1S-methylcarbamoyl-propyl)-N^4-hydroxy-2R-isobutyl-3S-methoxy-succinamide,

N^4-Hydroxy-2R-isobutyl-N^1-[2-mercapto-2-methyl-1S-methylcarbamoyl-propyl)-3S-methoxy-succinamide,

2S-Allyl-N^4-(2-Fluoro-2-methyl-1S-methylcarbamoyl-propyl)-N^1-hydroxy-3R-isobutyl-succinamide,

2S-Allyl-N^1-hydroxy-3R-isobutyl-N^4-(2-methyl-1S-methylcarbamoyl-2-phenyl-propyl)-succinamide,

2S-Allyl-N4-(2,2-dimethyl-1S-methylcarbamoyl-but-3-enyl)-N1-hydroxy-3R-isobutyl-succinamide,

3R-(3S-Hydroxycarbamoyl-2R-isobutyl-hex-5-enoylamino)-2,2,N-trimethyl-succinamic acid methyl ester,

3R-[2,2-Dimethyl-1S-(pyridin-2-ylcarbamoyl)-propylcarbamoyl]-2S-hydroxy-5-methyl-hexanoic acid,

and salts hydrates or solvates thereof.

As mentioned above, compounds of formula (I) are useful in human or veterinary medicine since they are active as inhibitors of MMPs, and a further advantage lies in their ability to inhibit the release of tumour necrosis factor (TNF) from cells.

Accordingly in another aspect, this invention concerns:

(i) a method of management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by MMPs and/or TNF in mammals, in particular in humans, which method comprises administering to the mammal an effective amount of a compound as defined with respect to formula (I) above, or a pharmaceutically acceptable salt thereof; and

(ii) a compound as defined with respect to formula (I) for use in human or veterinary medicine, particularly in the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by MMPs and/or TNF; and

(iii) the use of a compound as defined with respect to formula (I) in the preparation of an agent for the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by MMPs and/or TNF.

Diseases or conditions mediated by MMPs include those involving tissue breakdown such as bone resorption, inflammatory and neuroinflammatory diseases, dermatological conditions, solid tumour growth and tumour invasion by secondary metastases, and angiogenesis dependent diseases, in particular rheumatoid arthritis, osteoarthritis, periodontitis, gingivitis, corneal ulceration, solid tumour growth and tumour invasion by secondary metastases, neovascular glaucoma, multiple sclerosis, and psoriasis. Diseases or conditions mediated by TNF include inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infections, shock states, graft versus host reactions and autoimmune disease.

In a further aspect of the invention there is provided a pharmaceutical or veterinary composition comprising a compound of formula (I) together with a pharmaceutically or veterinarily acceptable excipient or carrier. Included within this aspect of the invention is a pharmaceutical or veterinary composition comprising a compound of formula (I) together with a pharmaceutically or veterinarily acceptable excipient or carrier, characterised in that the composition is adapted for oral administration.

One or more compounds of general formula (I) may be present in the composition together with one or more excipient or carrier.

The compounds with which the invention is concerned may be prepared for administration by any route consistent with their pharmacokinetic properties. The orally administrable compositions may be in the form of tablets, capsules, powders, granules, lozenges, liquid or gel preparations, such as oral, topical, or sterile parenteral solutions or suspensions. Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrrolidone; fillers for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricant, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be

coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

The dosage unit involved in oral administration may contain from about 1 to 250mg, preferably from about 25 to 250mg of a compound of the invention. A suitable daily dose for a mammal may vary widely depending on the condition of the patient. However, a dose of a compound of general formula I of about 0.1 to 300mg/kg body weight, particularly from about 1 to 100mg/kg body weight may be appropriate.

For topical application to the skin, the drug may be made up into a cream, lotion or ointment. Cream or ointment formulations which may be used for the drug are conventional formulations well known in the art, for example as described in standard textbooks of pharmaceutics such as the British Pharmacopoeia.

For topical application to the eye, the drug may be made up into a solution or suspension in a suitable sterile aqueous or non aqueous vehicle. Additives, for instance buffers such as sodium metabisulphite or disodium edeate; preservatives including bactericidal and fungicidal agents such as phenyl mercuric acetate or nitrate, benzalkonium chloride or chlorhexidine, and thickening agents such as hypromellose may also be included.

The dosage for topical administration will of course depend on the size of the area being treated. For the eyes, each dose may typically be in the range from 10 to 100mg of the drug.

The active ingredient may also be administered parenterally in a sterile medium. Depending on the vehicle and concentration used, the drug can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle.

For use in the treatment of rheumatoid arthritis, the drug can be administered by the oral route or by injection intra-articularly into the affected joint. The daily dosage for a 70kg mammal may be in the range 10mgs to 1gram.

The following examples describe the preparation of compounds of the invention. The preferred isomers of these compounds are isolated by standard chromatographic techniques.

Unless otherwise indicated, the amino acids used in the examples were commercially available or were prepared according to literature procedures. Preparation of the starting materials 2R-(2,2-dimethyl-5-oxo-[1,3]-dioxalan-4S-yl)-4-methyl-pentanoic acid and 2S-allyl-3R-isobutyl-succinic acid 1-*tert*-butyl ester has been described previously in WO 94/02447 and WO 94/21625, respectively.

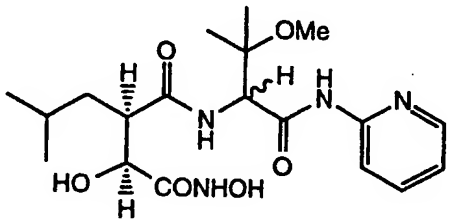
The following abbreviations have been used throughout:

DMF	N,N-Dimethylformamide
HOBt	1-Hydroxybenzotriazole
NMM	N-Methylmorpholine
THF	Tetrahydrofuran
TFA	Trifluoroacetic acid
TLC	Thin layer chromatography
EDC	N-Ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride

^{1}H and ^{13}C NMR spectra were recorded using a Bruker AC 250E spectrometer at 250.1 and 62.9 MHz, respectively. Elemental microanalyses were performed by Medac Ltd. (Department of Chemistry, Brunel University, Uxbridge, Middlesex UB8 3PH).

EXAMPLE 1

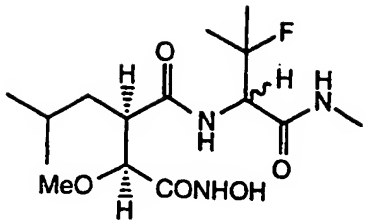
2S,N1-Dihydroxy-3R-isobutyl-N4-[2-methoxy-2-methyl-1RS-(pyridin-2-yl-carbamoyl)-propyl]-succinamide



Step A:

2R-(2,2-Dimethyl-5-oxo-[1,3]dioxolan-4S-yl)-4-methyl-pentanoic acid [2-methoxy-2-methyl-1RS-(pyridin-2-ylcarbamoyl)-propyl]-amide

To a solution of 2R-(2,2-dimethyl-5-oxo-[1,3]-dioxalan-4S-yl)-4-methyl-pentanoic acid (280 mg, 1.23 mmol) in DMF (80 ml) was added HOBt (199 mg, 1.48 mmol) and EDC (290 mg, 1.48 mmol). The reaction mixture was stirred for 2 hours at room temperature, RS-3-methoxyvaline N-(2-pyridyl)amide (260 mg, 1.23 mmol) was added and stirring was continued overnight. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, 3% methanol in dichloromethane) to give the desired product as a white foam (280 mg, 54%). ^{1}H-NMR; δ ($CDCl_3$), 8.58 (0.6H, d, J = 2.7 Hz), 8.56 (0.4H, d, J = 2.5 Hz), 8.35 (1H, m), 8.14 - 8.09 (1H, m), 7.30 - 7.25 (1H, m), 6.80 (1H, m), 4.67 -



STEP A:

2S-Hydroxy-3R-isobutyl-succinic acid dimethyl ester

2R-(2,2-Dimethyl-5-oxo-[1,3]-dioxolan-4S-yl)-4-methyl-pentanoic acid (75.0 g, 0.326 mol) was dissolved in methanol (500 ml) and cooled to 0°C and the resulting solution was saturated with hydrogen chloride gas. The reaction mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure and the residue was dissolved in dichloromethane and washed successively with saturated $NaHCO_3$ and brine. The organic layer was dried (anhydrous $MgSO_4$), filtered and evaporated to dryness under reduced pressure to give the title compound (53 g, 75%). ^{1}H-NMR; δ ($CDCl_3$), 4.10 (1H, d, J = 4.0 Hz), 3.60 (3H, s), 3.50 (3H, s), 2.82 - 2.74 (1H, m), 1.61 - 1.40 (2H, m) 1.33 - 1.23 (1H, m) and 0.76 - 0.73 (6H, m).

STEP B:

2R-Isobutyl-3S-methoxy-succinic acid dimethyl ester

2S-Hydroxy-3R-isobutyl-succinic acid dimethyl ester (9.6 g 44 mmol) was dissolved in DMF (5 ml) and distilled iodomethane (3.3 ml) and silver (I) oxide (11.2 g) were added. The reaction was stirred with the exclusion of light for 2 days at room temperature. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, dichloromethane) to give the title compound as a yellow viscous liquid, 4.70 g (46%). ^{1}H-NMR; δ

($CDCl_3$), 3.83 (1H, d, J = 7.5 Hz), 3.71 (3H, s), 3.62 (3H, s), 3.30 (3H, s), 2.89 - 2.80 (1H, m), 1.65 - 1.39 (2H, m), 1.15 - 1.04 (1H, m) and 0.83 - 0.81 (6H, m).

STEP C:

2R-Isobutyl-3S-methoxy-succinic acid dilithium salt

Lithium hydroxide (1.76 g, 42.0 mmol) was added to a solution of 2R-isobutyl-3S-methoxy-succinic acid dimethyl ester (4.70 g, 20.0 mmol) in methanol (30 ml) and water (30 ml). The reaction mixture was stirred at room temperature for 2 hours then solvents were removed under reduced pressure to give the product as a yellow solid (4.40 g, 100%). ^{1}H-NMR; δ (CD_3OD), 3.52 (1H, d, J = 5.1 Hz), 3.27 (3H, s), 2.69 - 2.61 (1H, m), 1.56 - 1.53 (2H, m), 1.34 - 1.28 (1H, m) and 0.82 - 0.78 (6H, m).

STEP D:

2R-Isobutyl-3S-methoxy-succinic acid 4-methyl ester

2R-Isobutyl-3S-methoxy-succinic acid dilithium salt (4.40 g, 20.0 mmol) was dissolved in THF (30 ml), the solution was cooled to 0°C and trifluoroacetic anhydride (30 ml) was added. The reaction was stirred for 4 hours, the solvent was removed under reduced pressure and the residue was dissolved in methanol (2 ml) at 0°C and stirred to room temperature overnight. The solvent was removed under reduced pressure to give the title compound as a yellow oil (7.0 g, including residual solvent), which was used without further purification in STEP E. ^{1}H-NMR; δ (CD_3OD), 7.61 (1H, d, J = 7.5 Hz), 3.65 (3H, s), 3.24 (3H, s), 2.78 - 2.67 (1H, m), 1.56 - 1.42 (2H, m), 1.09 - 1.03 (1H, m) and 0.81 - 0.79 (6H, m).

STEP E:

3R-(2-Fluoro-2-methyl-1RS-methylcarbamoyl-propylcarbamoyl)-2S-methoxy-5-methyl-hexanoic acid methyl ester

To an ice-cooled solution of 2R-isobutyl-3S-methoxy-succinic acid 4-methyl ester (1.5 g, 6.91 mmol) in DMF (35 ml) was added HOBt (1.07 g, 7.9 mmol) and EDC (1.51 g, 7.9 mmol) with stirring. The reaction mixture was allowed to warm slowly to room temperature and stirred for a further 2 hours to ensure complete formation of the activated ester, before cooling back to 0°C. In a separate vessel, 2RS-3-fluorovaline N-methylamide (0.97 g, 6.58 mmol) was prepared by mixing 2RS-3-fluorovaline N-methylamide trifluoroacetate salt with N-methylmorpholine (1.29 ml) in DMF (25 ml). The solution of free amine thus formed was added to the activated ester and the mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate and the solution was washed successively with 1M HCl, 1M Na_2CO_3 and brine. The organic layer was dried (anhyd. $MgSO_4$), filtered and evaporated to give the title compound as a 1:1 mixture of diastereoisomers (1.89 g, 73%) ^{1}H-NMR; δ ($CDCl_3$), 7.00 (0.5H, d, J = 8.9 Hz), 6.91 (0.5H, J = 9.0 Hz), 6.51 (0.5H, br s), 6.31 (0.5H, br s), 4.58 (1H, m), 3.88 (1H, m), 3.78 (1.5H, s), 3.77 (1.5H, s), 3.42 (1.5H, s), 3.41 (1.5H, s), 2.81 (3H, m), 2.76 (1H, m), 1.69 (1H, m), 1.55 (1H, m), 1.52 (1.5H, s), 1.50 (1.5H, s), 1.42 (1.5H, s), 1.35 (1.5H, s), 1.21 (1H, m) and 0.91 (6H, m).

STEP F:

3R-(2-Fluoro-2-methyl-1RS-methylcarbamoyl-propylcarbamoyl)-2S-methoxy-5-methyl-hexanoic acid lithium salt

3R-(2-Fluoro-2-methyl-1RS-methylcarbamoyl-propylcarbamoyl)-2S-methoxy-5-methyl-hexanoic acid methyl ester (1.89 g, 4.82 mmol) was dissolved in methanol (60 ml) and water (20 ml) and lithium hydroxide (212 mg, 5.06 mmol) was added. The mixture was stirred for 4 hours at room temperature whereupon hydrolysis was shown to be complete by TLC. The solvents were removed under reduced

pressure to leave an oil which was dried under high vacuum. Yield: 1.81 g (ca. quant.). ^{1}H-NMR; δ (CD_3OD), 4.39 (1H, m), 3.50 (1H, m), 3.20 (3H, m), 2.67 (3H, m), 1.61 (2H, m), 1.45 - 1.28 (6H, br m), 1.15 (1H, m) and 0.80 (6H, m).

STEP G:

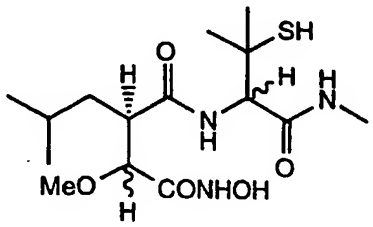
N^1-(2-Fluoro-2-methyl-1RS-methylcarbamoyl-propyl)-N^4-hydroxy-2R-isobutyl-3S-methoxy-succinamide

A solution of 3R-(2-fluoro-2-methyl-1RS-methylcarbamoyl-propylcarbamoyl)-2S-methoxy-5-methyl-hexanoic acid lithium salt (1.81 g, 4.71 mmol) in DMF was converted to the HOBt activated ester, using a procedure analogous to that described in STEP E. Hydroxylamine hydrochloride (492 mg, 7.07 mmol) and NMM (777µl, 7.07 mmol) were added and the reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure leaving an oil which was purified by column chromatography (acid-washed silica gel, gradient elution with 0 to 5% methanol in dichloromethane) to yield the title compound as a white foam (85 mg, 5%; 1:1 mixture of diastereoisomers). m.p. 173 - 175°C. ^{1}H-NMR; δ (CD_3OD), 4.49 (0.5H, s), 4.45 (0.5H, s), 3.46 (0.5H, d, J = 9.5 Hz), 3.23 (0.5H, m), 3.20 (1.5H, s), 3.15 (1.5H, s), 2.73 (1H, m), 2.65 (1.5H, s), 2.64 (1.5H, s), 1.51 (1H, m), 1.45 - 1.24 (7H, br m), 0.94 (1H, m) and 0.82 - 0.74 (6H, br m). ^{13}C-NMR; δ (CD_3OD), 175.5, 175.2, 171.0, 169.3, 169.2, 63.3, 63.0, 60.7, 59.3, 58.1, 38.4, 26.5, 25.5, 24.9, 24.6, 23.9, 21.9 and 21.6. IR; ν_{max} (KBr), 3306, 2957, 2442, 2360, 1638, 1539, 1456, 1387, 1212. 1154 and 1106 cm^{-1}. Found: C 50.58, H 7.78, N 11.70%; $C_{15}H_{28}FN_3O_5 \cdot 0.4\ H_2O$ requires: C 50.52, H 8.14, N 11.78%.

The following additional compound was prepared according to the methods of Example 2:

EXAMPLE 3

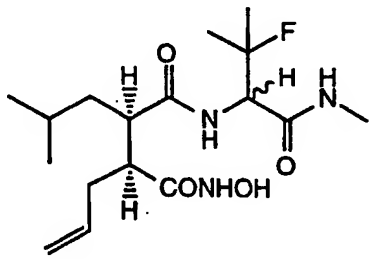
N^4-Hydroxy-2R-isobutyl-N^1-[2-mercapto-2-methyl-1RS-methylcarbamoyl-propyl)-3RS-methoxy-succinamide



Mixture of diastereoisomers. ^{1}H-NMR; δ ($(CD_3)_2SO$, major diastereoisomer), 10.68 (1H, s), 8.91 (1H, s), 7.80 (1H, d, J = 9.1 Hz), 7.15 (1H, m), 4.38 (1H, d, J = 4.9 Hz), 3.26 (1H, d, J = 4.0 Hz), 3.20 (3H, s), 2.75 (1H, m), 2.63 (1H, s), 2.45 (3H, d, J = 3.9 Hz), 1.28 (3H, s), 1.21 (3H, s), 1.40 - 1.10 (3H, br m) and 0.69 (6H, br m). ^{13}C-NMR; δ ($(CD_3)_2SO$, major diastereoisomer), 172.4, 169.5, 166.0, 81.0, 60.9, 47.8, 46.2, 37.2, 32.9, 30.8, 28.1, 25.5, 25.3, 23.9 and 21.9.

EXAMPLE 4

2S-Allyl-N^4-(2-Fluoro-2-methyl-1RS-methylcarbamoyl-propyl)-N^1-hydroxy-3R-isobutyl-succinamide



STEP A:

2S-[1R-(2-Fluoro-2-methyl-1RS-methylcarbamoyl-propylcarbamoyl)-3-methyl-

butyl]-pent-4-enoic acid *tert*-butyl ester

RS-3-fluorovaline TFA salt (2.12, 8.04 mmol) was dissolved in ethyl acetate (100 ml) and converted to the free amine by dropwise addition of NMM (1.58 ml). To this solution was added 2S-allyl-3R-isobutyl-succinic acid 1-*tert*-butyl ester dicyclohexylamine salt (3.98 g, 8.84 mmol), HOBt (1.30 g, 9.65 mmol) and EDC (1.85 g, 9.65 mmol). The mixture was heated at reflux for 8 hours and then left to stir overnight at room temperature. The precipitated was removed by filtration and the filtrate was washed successively with 1M HCl, 1M Na_2CO_3 and brine, dried over anhydrous $MgSO_4$ and filtered. The solvent was evaporated and the residue was purified by column chromatography to afford the title compound (1.18 g, 37%; 2:1 mixture of diastereoisomers). ^{1}H-NMR; δ ($CDCl_3$), 6.82 (1H, t, J = 8.9 Hz), 6.61 (1H, m), 5.71 (1H, br m), 5.04 (2H, m), 4.78 (1H, m), 2.89 (1H, d, J = 6.9 Hz), 2.85 (2H, d, J = 6.9 Hz), 2.55 (2H, m), 2.21 (2H, m), 1.75 (1H, m), 1.52 (1H, s), 1.46 (6H, s), 1.42 (3H, s), 1.40 (2H, s), 1.39 (2H, s), 1.31 (1H, s), 1.12 (1H, m) and 0.89 (6H, m).

STEP B:

2S-[1R-(2-Fluoro-2-methyl-1RS-methylcarbamoyl-propylcarbamoyl)-3-methyl-butyl]-pent-4-enoic acid

2S-[1R-(2-Fluoro-2-methyl-1RS-methylcarbamoyl-propylcarbamoyl)-3-methyl-butyl]-pent-4-enoic acid *tert*-butyl ester (1.18 g, 2.94 mmol) was dissolved in dichloromethane (50 ml) and TFA (50 ml) and the solution was stored overnight at 0°C. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting foam (1.07 g, including residual TFA) was used without further purification. ^{1}H-NMR; δ ($CDCl_3$), 5.62 (1H, m), 4.90 (2H, m), 4.51 (1H, br m), 2.66 (1H, s), 2.64 (2H, s), 2.60 (1H, m), 2.46 (1H, m), 2.27 - 2.05 (2H, br m), 1.60 (1H, m), 1.39 (2H, s), 1.36 (1H, s), 1.35 (1H, m), 1.30 (2H, s), 1.28 (1H, s), 1.05 (1H, m) and 0.86 (6H, br m).

STEP C:

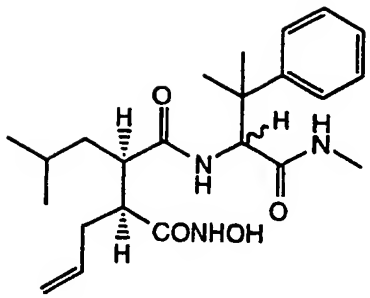
2S-Allyl-N4-(2-fluoro-2-methyl-1RS-methylcarbamoyl-propyl)-N1-hydroxy-3R-isobutyl-succinamide

2S-[1R-(2-Fluoro-2-methyl-1RS-methylcarbamoyl-propylcarbamoyl)-3-methyl-butyl]-pent-4-enoic acid (1.07 g, 3.11 mmol) was converted to the title compound by a method analogous to that described in Example 2. After solvent evaporation the product was isolated by trituration with ethyl acetate and water. The resulting white solid was collected by filtration, washed successively with ethyl acetate and diethyl ether and dried under high vacuum. Yield: 397 mg (35%; ca. 2:1 mixture of diastereoisomers). ^{1}H-NMR; δ (CD_3OD), 10.34 (1H, s), 8.64 (1H, br s), 8.23 (0.65H, d, J = 9.4 Hz), 8.11 (0.35H, d, J = 9.2 Hz), 7.86 (1H, m), 5.39 (1H, m), 4.74 (2H, m), 4.43 (1H, m), 2.57 (2H, m), 2.44 (3H, d, J = 3.9 Hz), 2.01 (1.3H, m), 1.78 (0.7H, m), 1.30 - 0.99 (8H, m and 4s) and 0.85 - 0.59 (7H, br m). ^{13}C-NMR; δ (CD_3OD), 171.5, 169.1, 168.5, 135.9, 115.8, 95.6, 94.3, 63.0, 58.6, 54.4, 52.2, 46.1, 45.5, 41.9, 36.2, 34.2, 25.2, 25.0, 24.3, 21.5 and 15.4. IR; ν_{max} (KBr), 3315, 2952, 1639, 1561, 1531, 1470, 1384, 1219 and 1150 cm^{-1}.

The following additional compounds were prepared according to the methods of Example 4:

EXAMPLE 5

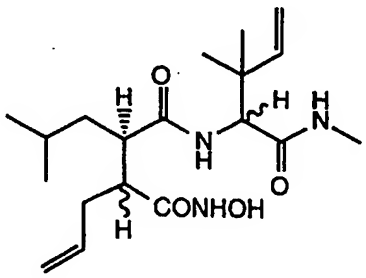
2S-Allyl-N1-hydroxy-3R-isobutyl-N4-(2-methyl-1RS-methylcarbamoyl-2-phenyl-propyl)-succinamide



White solid (1:1 mixture of diastereoisomers). m.p. 206 - 209 °C. ^{1}H NMR; δ ($CDCl_3$), 7.39 - 7.29 (2H, m) 7.24 - 6.99 (3H, m), 5.59 - 5.24 (1H, m), 4.90 - 4.68 (3H, m), 2.53 (1.5H, s), 2.51 (1.5H, s), 2.50 - 2.33 (1H, m), 2.21 - 1.63 (3H, m), 1.38 (1.5H, s), 1.37 (1.5H, s), 1.30 - 1.10 (2H, m), 1.00 - 0.82 (1H, m), 0.74 (1.5H, d, J = 6.4 Hz), 0.68 (1.5H, J = 6.5 Hz) and 0.60 - 0.50 (3H, m). ^{13}C NMR; δ ($CDCl_3$), 176.2, 176.1, 172.8, 172.6, 172.5, 147.6, 136.3, 136.2, 129.3, 129.2, 127.5, 127.3, 117.3, 62.6, 62.5, 48.0, 47.7, 47.4, 41.8, 41.1, 35.9, 35.6, 27.6, 27.3, 26.3, 26.1, 24.5, 24.4 and 21.6. IR; ν_{max} (KBr), 3274, 2956, 1634, 1541, 1388, 1369 cm^{-1}.

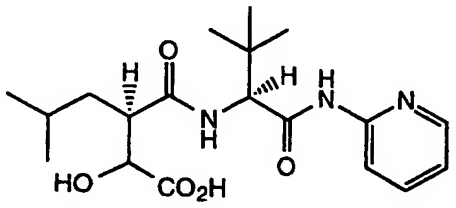
EXAMPLE 6

2RS-Allyl-N^4-(2,2-dimethyl-1RS-methylcarbamoyl-but-3-enyl)-N^1-hydroxy-3R-isobutyl-succinamide



White solid (mixture of diastereoisomers). m.p. 208 - 210°C. ^{1}H NMR; δ (CD_3OD),

methyl-hexanoic acid



STEP A:

2-(2,2-Dimethyl-5-oxo-[1,3]dioxolan-4-yl)-4-methyl-pentanoic acid [2,2-dimethyl-1-(pyridin-2-ylcarbamoyl)-propyl]-amide

The title compound was prepared from 2R-(2,2-dimethyl-5-oxo-[1,3]-dioxalan-4S-yl)-4-methyl-pentanoic acid and S-*tert*-leucine N-(2-pyridyl)amide according to the method described in Example 1 STEP A. ^{1}H-NMR; δ ($CDCl_3$), 9.54 (1H, s), 8.49 (1H, m), 8.21 (1H, d, J = 8.4 Hz), 7.72 (1H, m), 7.10 (1H, m), 6.82 (1H, d, J = 9.1 Hz), 4.58 (1H, d, J = 5.8 Hz), 4.57 (1H, d, J = 9.2 Hz), 2.80 (1H, m), 1.80 (1H, m), 1.70 - 1.58 (5H, m and s), 1.54 (3H, s), 1.01 (9H, s), 0.92 (3H, d, J = 6.6 Hz) and 0.89 (3H, d, J = 6.5 Hz).

STEP B:

3R-[2,2-Dimethyl-1S-(pyridin-2-ylcarbamoyl)-propylcarbamoyl]-2-hydroxy-5-methyl-hexanoic acid

2-(2,2-Dimethyl-5-oxo-[1,3]dioxolan-4-yl)-4-methyl-pentanoic acid [2,2-dimethyl-1-(pyridin-2-ylcarbamoyl)-propyl]-amide (500 mg, 1.15 mmol) was suspended in methanol (8 ml) and water (6 ml) and stirred until a clear solution was obtained (7 days). TLC indicated that all of the starting material had been consumed. The solvents were removed under reduced pressure to leave the title compound as a

white solid (450 mg, ca. quant.). m.p. 108.5 - 110°C. ^{1}H-NMR; δ ($CDCl_3$), 8.82 (1H, d, J = 8.8 Hz), 8.41 (1H, m), 8.27 (1H, m), 7.45 (1H, m), 7.07 (1H, d, J = 7.9 Hz), 4.44 (1H, m), 4.42 (1H, d, J = 3.1 Hz), 2.97 (1H, m), 1.72 (3H, m), 1.12 (9H, s), 0.98 (3H, d, J = 5.7 Hz) and 0.96 (3H, d, J = 5.8 Hz). ^{13}C-NMR; δ ($CDCl_3$), 174.1, 173.4, 171.7, 147.7, 146.5, 138.1, 134.8, 120.1, 117.5, 71.3, 63.0, 48.0, 38.4, 34.5, 27.1, 25.6 and 22.5.

2S,N1-Dihydroxy-3R-isobutyl-N4-[2-methoxy-2-methyl-1S-(pyridin-2-yl-carbamoyl)-propyl]-succinamide,

N1-(2-Fluoro-2-methyl-1S-methylcarbamoyl-propyl)-N4-hydroxy-2R-isobutyl-3S-methoxy-succinamide,

N4-Hydroxy-2R-isobutyl-N1-[2-mercapto-2-methyl-1S-methylcarbamoyl-propyl)-3S-methoxy-succinamide,

2S-Allyl-N4-(2-Fluoro-2-methyl-1S-methylcarbamoyl-propyl)-N1-hydroxy-3R-isobutyl-succinamide,

2S-Allyl-N1-hydroxy-3R-isobutyl-N4-(2-methyl-1S-methylcarbamoyl-2-phenyl-propyl)-succinamide,

2S-Allyl-N4-(2,2-dimethyl-1S-methylcarbamoyl-but-3-enyl)-N1-hydroxy-3R-isobutyl-succinamide,

3R-(3S-Hydroxycarbamoyl-2R-isobutyl-hex-5-enoylamino)-2,2,N-trimethyl-succinamic acid methyl ester, or

3R-[2,2-Dimethyl-1S-(pyridin-2-ylcarbamoyl)-propylcarbamoyl]-2S-hydroxy-5-methyl-hexanoic acid,

or a salt hydrate or solvate thereof.

INTERNATIONAL SEARCH REPORT

Int onal Application No
PCT/GB 95/01698

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D213/75 C07C259/06 C07C323/60

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,94 10990 (BRITISH BIO-TECHNOLOGY LTD., UK) 26 May 1994 see claims ---	1,2
A	EP,A,0 497 192 (HOFFMANN-LA ROCHE, F., UND CO. A.-G., SWITZ.) 5 August 1992 cited in the application see claims ---	1,2
A	WO,A,94 21625 (BRITISH BIO-TECHNOLOGY LTD., UK) 29 September 1994 cited in the application see claims ---	1,2
A	GB,A,2 268 934 (BRITISH BIO TECHNOLOGY) 26 January 1994 see claims --- -/--	1,2

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
28 February 1996	-8. 03 96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Authorized officer Seufert, G

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INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/GB 95/01698

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO,A,95 19961 (BRITISH BIOTECH PHARMACEUTICALS LTD., UK) 27 July 1995 cited in the application see claims -----	1,2

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int .onal Application No
PCT/GB 95/01698

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9410990	26-05-94	AU-B-	5430194	08-06-94
		EP-A-	0667770	23-08-95
EP-A-0497192	05-08-92	AU-B-	658387	13-04-95
		AU-B-	1025792	06-08-92
		CA-A-	2058797	02-08-92
		JP-A-	4352757	07-12-92
		NZ-A-	241409	27-06-94
		SI-A-	9210090	30-04-95
		US-A-	5304549	19-04-94
WO-A-9421625	29-09-94	AU-B-	6213194	11-10-94
		CA-A-	2158352	29-09-94
		EP-A-	0689538	03-01-96
		FI-A-	954351	15-09-95
		GB-A-	2290543	03-01-96
		NO-A-	953652	15-09-95
GB-A-2268934	26-01-94	AU-B-	4715293	14-02-94
		AU-B-	661410	20-07-95
		AU-B-	4715393	14-02-94
		CA-A-	2140626	03-02-94
		CZ-A-	9500157	18-10-95
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		EP-A-	0651738	10-05-95
		EP-A-	0651739	10-05-95
		FI-A-	950262	20-01-95
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		WO-A-	9402447	03-02-94
		GB-A-	2268933	26-01-94
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		ZA-A-	9305351	14-02-94
		ZA-A-	9305352	16-05-94

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int onal Application No
PCT/GB 95/01698

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9519961	27-07-95	AU-B- 1460395	08-08-95

Form PCT/ISA/210 (patent family annex) (July 1992)